GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: June 15, 1979

Project Title: Studies on Fatty ACYL COA Dehydrogenase and ETF

Project No: G-33-K02 Green card

Project Director: Dr. Carole L. Hall

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, MD 20014

Agreement Period: From 7/1/79 Until 6/30/80 (02 year)

Type Agreement: Grant No. 5 R01 GM25494-02

Amount: $60,247 PHS Funds (G-33-K02) PHS Direct Costs: $38,496
3,730 GIT Contribution (G-33-343)
$63,977 Total

Reports Required: Annual Progress Reports with Continuation Applications; Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

Technical Matters

Program Official
Arthur E. Heming, Ph.D.
Associate Director for Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

Program Administrator:
Dr. Eugene J. Oliver

Phone: 301-496-7518

Contractual Matters
(thru OCA)

Grants Management Official
Ms. Evelyn W. Carlin
Grants Management Officer
Office of Associate Director for Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

Grants Management Specialist:
Ms. Ruth C. Monaghan

301-496-7746

NOTE: FOLLOW-ON PROJECT TO G-33-K01 (01 YEAR)

Defense Priority Rating: None

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director—EES
- Accounting Office
- Procurement Office
- Security Coordinator (OCA)
- Reports Coordinator (OCA)
- Library, Technical Reports Section
- EES Information Office
- EES Reports & Procedures
- Project File (OCA)
- Project Code (GTRI)
- Other

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 4/3/81

Project Title: Studies on Fatty ACYL COA Dehydrogenase and ETF

Project No: G-33-K02

Project Director: Dr. Carole L. Hall

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences;
5 R01 GM25494-02

Effective Termination Date: 6/30/80 (end of 02 year)

Clearance of Accounting Charges: ---

Grant/Contract Closeout Actions Remaining:

NONE

- _ Final Invoice and Closing Documents
- _ Final Fiscal Report
- _ Final Report of Inventions
- _ Govt. Property Inventory & Related Certificate
- _ Classified Material Certificate
- _ Other _____

NOTE: Continued by G-33-K03

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

Administrative Coordinator
Research Property Management
Accounting Office
Procurement Office
Research Security Services
Reports Coordinator (OCA)
Legal Services (OCA)
Library, Technical Reports
EES Research Public Relations (2)
Project File (OCA)
Other: _____

G-33-K02

GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GEORGIA 30332

OFFICE OF
THE
COMPTROLLER

October 14, 1980

Grants Management Officer
Office of Associate Director
for Program Activities
National Institute of General
Medical Sciences
DHHS/PHS/NIH
Bethesda, Maryland 20205

Dear Sir or Madam:

Enclosed is the Report of Research Grant Expenditures (Form HEW-489) for Grant No. 5 R01 GM25494-02 covering the period 7/1/79 - 6/30/80.

If you have any questions or require additional information, please let us know.

Sincerely,

David V. Welch

David V. Welch, Manager
Grants and Contracts Accounting

DVW/BITS/jb
Enclosure
cc: Dr. C. L. Hall
Dr. J. A. Bertrand
Mr. H. Dean
Mr. O. H. Rodgers
File G-33-K02

DEPARTMENT OF HEALTH AND HUMAN SERVICES

(Instructions are on reverse)

Grant No.
5 R01 GM25494-02

DATE OF THIS REPORTING PERIOD
FROM 7/1/79 TO 6/30/80

PROJECT PERIOD
FROM 7/1/78 TO 6/30/81

☐ CHECK IF FINAL REPORT

NAME AND ADDRESS OF GRANTEE INSTITUTION

Georgia Institute of Technology
Atlanta, Georgia 30332

TRANSACTION NO.
(08)RIGM 25494A

INSTITUTIONAL ID NO.
G-33-K02

1. Expenditures of DHHS Funds for this Reporting Period

a. Personnel	$	h. Alterations and renovations	
b. Consultant services		i. Other	
c. Equipment			
d. Supplies		j. Total direct costs	38,496.00
e. Travel, domestic		k. Indirect costs: Rate 76 % ☑ S&W ☐ TDC	
f. Travel, foreign		Base $ 26,645.27	20,250.41
g. Patient care costs		l. TOTAL	$ 58,746.41

2. Expenditures from Prior Periods (previously reported)	62,405.61
3. Cumulative Expenditures	121,152.02
4. Total Amount Awarded — Cumulatively	127,292.00
5. Unexpended Balance (Item 4 less Item 3)	6,139.98
6. Unliquidated Obligations	-0-
7. Unobligated Balance (Item 5 less Item 6)	6,139.98
8.a. Cost Sharing Information — Grantee Contribution This Period	3,730.20
b. % of Total Project Costs (Item 8a divided by total of Items 1 and 8a)	% 6.0
9.a. Interest/Income (enclose check)	-0-
b. Other Refundable Income (enclose check)	-0-

10. Remarks

I hereby certify that this report is true and correct to the best of my knowledge, and that all expenditures reported herein have been made in accordance with appropriate grant policies and for the purposes set forth in the application and award documents.

Carole L. Hall
Dr. C. L. Hall — Research Scientist — 10/3/80
SIGNATURE — Date

David V. Welch
SIGNATURE OF INSTITUTION OFFICER — 10/14/80
DATE

David V. Welch, Mgr., Grants & Contracts Acctg.
404/894-4624

Formerly HE-489

REPORT OF RESEARCH GRANT EXPENDITURES

G-33-K.2

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT	1 R01 GM 25494-03	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
Hall, Carole L.	FROM	THROUGH
NAME OF ORGANIZATION		
Georgia Institute of Technology	July 1, 1980	June 30, 1981

TITLE (Repeat title shown in Item 1 on first page)

Fatty Acyl CoA Dehydrogenase and ETF

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1(b) 1.Carole L. Hall, Federation Proceeding 1980," Dehydrogenation of Acyl CoA Dehydrogenase (GAD) and ETF "(in press).

2.Carole L. Hall, 14th Annual Regional Lipid Conference, Knoxville, Tenn.(1979) "A New Specific Assay for ETF and ETF-linked acyl CoA Dehydrogenases."

3.Carole L. Hall and J. David Lambeth, J. Biol. Chem., "Electron Transfer from Fatty Ac CoA to General Acyl CoA Dehydrogenase and Electron Transfer Flavoprotein," in press., due April 25,1980

4.Carole L. Hall, Methods in Enzymology, Lipids (J.M. Lowenstein Ed.) "Acyl CoA Dehydrogenases from Pig Liver Mitochondria (in press)

5.Carole L. Hall, Methods in Enzymology, Lipids (J. M. Lowenstein, Ed. (in press). "ETF from Pig Liver Mitochondria" (in press).

(c) 1.Carole L. Hall, submitted to J. Biol. Chem., "On the Dehydrogenation of Acyl CoA by General Acyl CoA Dehydrogenase and Electron Transfer Flavoprotein".

2.Carole L. Hall, M.S. in preparation "Isovaleryl CoA Dehydrogenase, A New Electron Transfer Flavoprotein-linked Flavoenzyme."

3.Carole L. Hall, "Assay of Electron Transfer Flavoprotein and ETF Linked Dehydrogenasesby Fluorescence Measurements." (M.S. in Preparation).

2. Professional Personnel

Changes: Teresa M. DeTar left voluntarily.
Eric N. Juberg was hired to fill the position vacated by T. M. DeTar.

Changes in Effort: Carole L. Hall taught at Georgia Tech during 1979-1980 and effort was reduced to 95%. Compensation was provided by Georgia Tech.

3. The long term goal is to elucidate the interaction of the two flavoproteins required in α,β-dehydrogenation of fatty acyl CoA's in β oxidation with each other, with the substrate and products of the dehydrogenation and with the rest of the β-oxidation enzymes, and to determine the mechanisms of the electron transfers involved and fate of product. This is being approached by rapid reaction and anaerobic titration techniques (utilizing the characteristic absorbance and fluorescences of those enzymes) displacement studies, and studies of catalytic activity in dye-complexed systems.

The aims of the research are:
(a) to determine if 2 é to 1 é transduction occurs on the dehydrogenase, between the two flavoproteins, on the ETF or at some other site (e.g., interaction

Section IV Continued

with the electron transport chain.

(b) To probe the relationship between these flavoproteins and the rest of the β-oxidation enzymes as possible effectors of the dehydrogenation.

(c) To determine the redox properties of the two flavoproteins both separately and together.

(d) To probe the nature of the apparent oxidized acyl CoA complex (green) of the short chain enzyme by attempting to displace and replace the complexing agent and to probe the postulated effects of hypoglycemic agents.

(e) To identify additional flavoproteins which have been isolated and interact with acyl CoA derivatives.

I have continued to investigate absorbance changes during catalysis to try to ascertain the mechanism of electron flow through this system. Some of the results are summarized in the following abstract accepted for presentation at the June 1980 ASBC/BS meeting in New Orleans, La.

DEHYDROGENATION OF ACYL CoA BY GENERAL ACYL CoA DEHYDROGENASE (G-AD) AND ETF. C. L. Hall* (SPON: D. H. Hall). School of Chemistry, Georgia Tech, Atlanta, Ga. 30332

Medium chain acyl CoA's bind rapidly and tightly to G-AD and rapidly (100 sec^{-1}) bleach the flavin. G-AD-C_8CoA complex reduces ETF first to anionic semiquinone (6 x 10^6 M^{-1} sec^{-1}) and then to the fully reduced form (5 x 10^5 M^{-1} sec^{-1}). Substrate-reduced ETF is reoxidized only slowly by air. Spectra of ETF observed during turnover with catalytic amounts of G-AD and 4-fold molar excesses of C_8CoA per ETF flavin showed changes in the UV and near UV which could be correlated with visible absorbance changes known to reflect redox states of ETF flavin. All of the ETF was converted to the anionic semiquinone (increase at 380 nm) and fully reduced (decrease at 380 nm) forms with rate constants virtually identical to those seen previously. Absorption at 320 nm increased with a rate constant similar to that for formation of fully reduced ETF, and decreased only as the flavin absorption at 440 nm returned. Increased absorbance at 280 nm (expected to indicate free enoyl CoA) appeared only as the 320 nm absorbance decreased. Changes at 280 nm showed that all of the substrate was dehydrogenated. The data suggest that G-AD-C_8CoA complex interacts with ETF to transfer one electron to the ETF flavin and then a new complex (represented by A_{320}) is formed more slowly with transfer of the second electron. This complex can not include G-AD but seems to involve reduced ETF and acyl CoA since the enoyl CoA is not released until the ETF is reoxidized.
(Supported by USPHS grant #GM 25494).

Section IV Continued

I have also purified and characterized a new ETF-linked flavoprotein which dehydrogenates isovaleryl CoA, a function previously thought to be carried out by general (G-AD) or short chain (SC-AD) acyl CoA dehydrogenases. Instead these highly purified enzymes do not utilize catalytically or form spectrally identifiable complexes with this branched-chain acyl CoA derived from amino acid catabolism. A manuscript describing the properties of this enzyme is in preparation and expected to be ready for submission shortly.

Other experiments involving this enzyme were and are still being carried out in collaboration with Dr. William Rhead of The University of Iowa Medical Center, Ames, Iowa. A manuscript describing the work is in preparation. In addition, Dr. Stephen Goodman of the University of Colorado Medical School, Denver, Colorado has initiated collaborative efforts to try to detect ETF in liver of human victims of as yet uncharacterized organic acidemias and acidurias. I have recently developed new specific, quantitative and fairly sensitive methods of measuring ETF fluoriometrically in crude samples. A preliminary report of these methods was made in Sept. 1979 at the Regional Lipids Conference in Nashville, Tenn. A manuscript describing the results is in preparation.

A series of experiments probing the mechanism of phenazine methosulfate action as an electron acceptor for acyl CoA dehydrogenase has begun, using both absorption and EPR Spectroscopy (in collaboration with a student, Mr. Mitchell Gould and Drs. B. Yamanashi and S. Lerman of the Department of Opthamology, Emory University Medical Center. Preliminary experiments probing the usefulness of pyocyanine (reduced by lipoyldehydrogenase and NaDH) as a reductant for ETF were positive. This is the first instance of ETF being reduced by anything other than reduced acyl CoA dehydrogenase. The use of these dye systems will potentially permit redox measurements of the acyl CoA dehydrogenase-substrate complex and of the ETF.

Finally, preliminary experiments to try to probe the nature of the acyl CoA dehydrogenase-substrate complex by EPR have begun, again in collaboration with Mr. Gould and Drs. Yamanashi and Lerman.

The research goals for the coming year are:

a) To study the formation and breakdown of the putative reduced ETF-enoyl CoA complex using different chain length (and branched chain) substrates and different dehydrogenases (short chain, isovaleryl).

b) To test the utility of the fluorescence assay for ETF using rat liver and human autopsy liver mitochondria, as well as other tissues as available.

c) To probe electron flow between acyl CoA dehydrogenase and ETF with emphasis on redox potentials and stoichiometry.

d) To continue to probe the acyl CoA dehydrogenase substrate complex using PMS and EPR spectroscopy

Section IV Continued

e) To continue in attempts to displace and identify the "greening" ligand of short chain acyl CoA dehydrogenase and to synthesiz possible ligands.

f) To further study the putative "β-oxidase complex" of pig liver mitochondria and study its interaction with acyl CoA dehydrogenase and/or ETF.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

4/23/80
Date

Carole L. Hall
Carole L. Hall, Principal Investigator